NUCLEO-CYTOPLASMIC INTERACTION DURING CONJUGATION IN TETRAHYMENA [1]

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Studies on the ciliated Protozoa, particularly *Paramecium aurelia* (Sonneborn, 1950b, 1951), have provided much information on the roles of the nucleus and the cytoplasm in cellular differentiation. One important observation in these studies (Sonneborn, 1947) is that the cytoplasm may control the kind of macronucleus developed in a cell; specifically, the cytoplasm may determine whether a new macronucleus will differentiate so as to control one or the other of the possible mating types. It has been suggested (Nanney, 1953) that the cytoplasm in these cells has been determined by the kind of macronucleus previously occupying the cell. This does not detract from the importance of the cytoplasm in cellular heredity, but emphasizes the importance of nucleo-cytoplasmic *interactions*.

Related to this problem of what determines the *kind* of macronucleus to develop is the problem of what determines *whether* a particular nucleus will differentiate into a macronucleus. In many ciliates the fertilization nucleus produced at nuclear reorganization divides twice to produce four presumably identical nuclei: two of these differentiate as macronuclei and two as micronuclei. Long ago Maupas (1889) suggested that this difference in the development of nuclei was due to localized differences in the cytoplasm surrounding the nuclei at a critical time in their development. Maupas based this suggestion on observations made on a group of ciliates, including particularly *Colpidium, Leucophrys* and *Glaucoma*. He observed that in these organisms the fertilization nucleus divided twice and that the spindles for the second post-zygotic division were oriented in such a fashion that two of the four division products in each cell were placed at the extreme anterior end of the cell and two were placed at the extreme posterior end of the cell. Those which were placed at the anterior end of the cell were observed to enlarge and become the new macronuclei while those at the posterior end remained small and became the new micronuclei. Here was a clear visible correlation between the location of a nucleus in the cytoplasm and its subsequent development. Although this correlation strongly suggested cytoplasmic control of nuclear development, other interpretations were possible and were not excluded.

A number of observations similar to those of Maupas have been made on a variety of organisms since Maupas' time and recently Sonneborn (1951) has directed attention to the nuclear events at conjugation in *Paramecium* where several additional instances of apparent cytoplasmic control of nuclear activity are

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found. The present study is presented as an extension of his observations to another organism, with certain experimental data bearing on his conclusions.

Materials and Methods

Strains of *Tetrahymena* were recently obtained (Elliott and Nanney, 1952) which permitted further analysis of some of these problems. These strains, designated as the AA strains, undergo conjugation regularly. The entire process has been analyzed both to gain insight into the factors controlling nuclear development and to provide a firm basis for subsequent genetic studies. The eight strains studied were all collected in the vicinity of Ann Arbor, Michigan and in each of them any isolated cell will give rise to a culture in which conjugation (selfing) occurs. However, no conjugants have been found which give rise to viable progeny. More recently (Elliott and Gruchy, 1952), other strains of *Tetrahymena* were collected near Woods Hole, Massachusetts, and in these strains—designated as the WH strains—Elliott has demonstrated that mating types exist and that viable progeny are produced. The details of conjugation in the WH strains resemble closely those in the AA strains with the exception of a few modifications related to the occurrence of mating types and a high frequency of spontaneous anomalies in the cytogenetic processes. These differences and their significance will be discussed in a later paper.

The selfing strains are designated as AA 1–8, and have been assigned to the genus *Tetrahymena* by Corliss and Furgason (in Elliott and Nanney, 1952). They show certain differences from the strains of *T. pyriformis* (=*geleii*) extensively studied and are, hence, described simply as *T. sp.* One of the principal differences between these strains and the long-maintained laboratory cultures of *T. pyriformis* lies in the fact that the latter are lacking in micronuclei. *T. sp.* has, as a rule, a single micronucleus, but occasional cells with as many as four micronuclei have been observed.

Most of these strains, perhaps all, can be grown on a defined medium (Elliott and Nanney, 1952), but in the present study all the cells were maintained in bacterized cultures. The medium was prepared by boiling 1½ grams of Cerophyl in a liter of distilled water, filtering and autoclaving. The day before the medium was to be used, it was inoculated with *Aerobacter aerogenes*. The general culture methods followed closely those described for *Paramecium* by Sonneborn (1950a).

Conjugation occurs regularly in all the stocks soon after the nutrient in the medium is exhausted. It has not been possible even after many serial isolations to derive cultures differing in mating type; any isolation gives rise to a clone within which nearly 100% conjugation can occur. No evidence was found for autogamy or any other process of nuclear reorganization which might account for diversities within a culture; hence, it seems reasonable to assume that the cells which conjugate are genetically alike. The question of whether the cells are of different mating types will be discussed in a later paper, but at the present time no evidence is available for any differences between the cells that conjugate.

Under all conditions thus far tried, the exconjugants die—usually without separating. The cultures are perpetuated from the individuals which have failed to conjugate. Attempts to obtain viable conjugants by growing cells from different sources in the same container and isolating conjugants have proved unsuccessful.

Since conjugation occurs within a single culture, it is difficult to control its initiation. Pairs are formed over a period of several hours and samples removed at any one time contain pairs in many stages of conjugation. The sequence of stages must, therefore, be inferred rather than directly demonstrated. Similarly the length of time necessary for the completion of the various stages cannot be determined readily.

In preparation for cytological studies, pairs were killed and fixed in hot Schaudinn's solution. Various staining techniques were used: these include the borax-carmine method of Dippell (in Sonneborn, 1950a), Dippell and Chao's (in Sonneborn, 1950a) modification of the De Lamater stain and the Giemsa method described by Preer (1950).

The figures representing the sequence of stages are camera lucida drawings of stained pairs. No attempt has been made to simulate the structural details of the nuclei or to indicate the precise number or size of the chromosomes. The chromosomes are small, numerous and difficult to count or draw, though this should be possible eventually. Characteristic changes may be noted in the staining properties of the chromosomes and these will be described later.

Descriptive

1. *The normal pattern of nuclear behavior*

The normal pattern of nuclear behavior during conjugation closely resembles that reported by Maupas (1889) for *Leucophrys patula*. Recent systematic revisions (Furgason, 1940; Corliss, 1952) indicate that *Leucophrys patula* is more correctly termed *Tetrahymena patula*, a species closely related to *T. sp.*; hence, this similarity in nuclear behavior is not surprising. In spite of the similarity in the cytogenetic details reported by Maupas and those reported below, it appears advisable to present briefly the normal sequence of events as a basis for comparison with the anomalies to be presented subsequently.

Conjugating cells attach at their oral surfaces (near the anterior ends of the cells) with a "face to face" orientation. Preliminary clumping reactions and nonspecific attachments have not been observed, but it is possible that these occur. At the time the cells first become attached, the single micronucleus is found near the macronucleus but may be anterior, posterior or lateral to it. Shortly after the initiation of conjugation the micronucleus moves into a region just anterior to the macronucleus (Fig. 1A), enlarges and begins to elongate into the typical "crescent" stage of the first pre-zygotic division (Fig. 1B). This crescent shortens in the later stages of this division (Fig. 1C) and the chromosomes become clearly visible. The first division is completed and the two daughter nuclei enter immediately into the second pre-zygotic division (Fig. 1D) still anterior to the macronucleus. During this division the chromosomes are much less prominent than during the previous division. Extrapolating from information on other ciliates whose genetics are well known (see Sonneborn, 1947), it appears probable that these first two divisions are the meiotic divisions and that the resulting four nuclei are haploid.

At the end of the second pre-zygotic division the four nuclei in each cell continue to migrate anteriorly until one of the nuclei comes in contact with the membrane between the cells and appears to attach to it. More specifically, the nuclei attach on the right side of the cell. The attachment of nuclei on opposite sides of the contact

surfaces is usually, but not always, synchronous. Following the attachment of one of the nuclei, the remaining nuclei in the cell begin to move posteriorly (Fig. 1E *et seq.*), eventually to disintegrate. These "relic" nuclei may persist for a variable length of time, but have never been observed to undergo any further divisions.

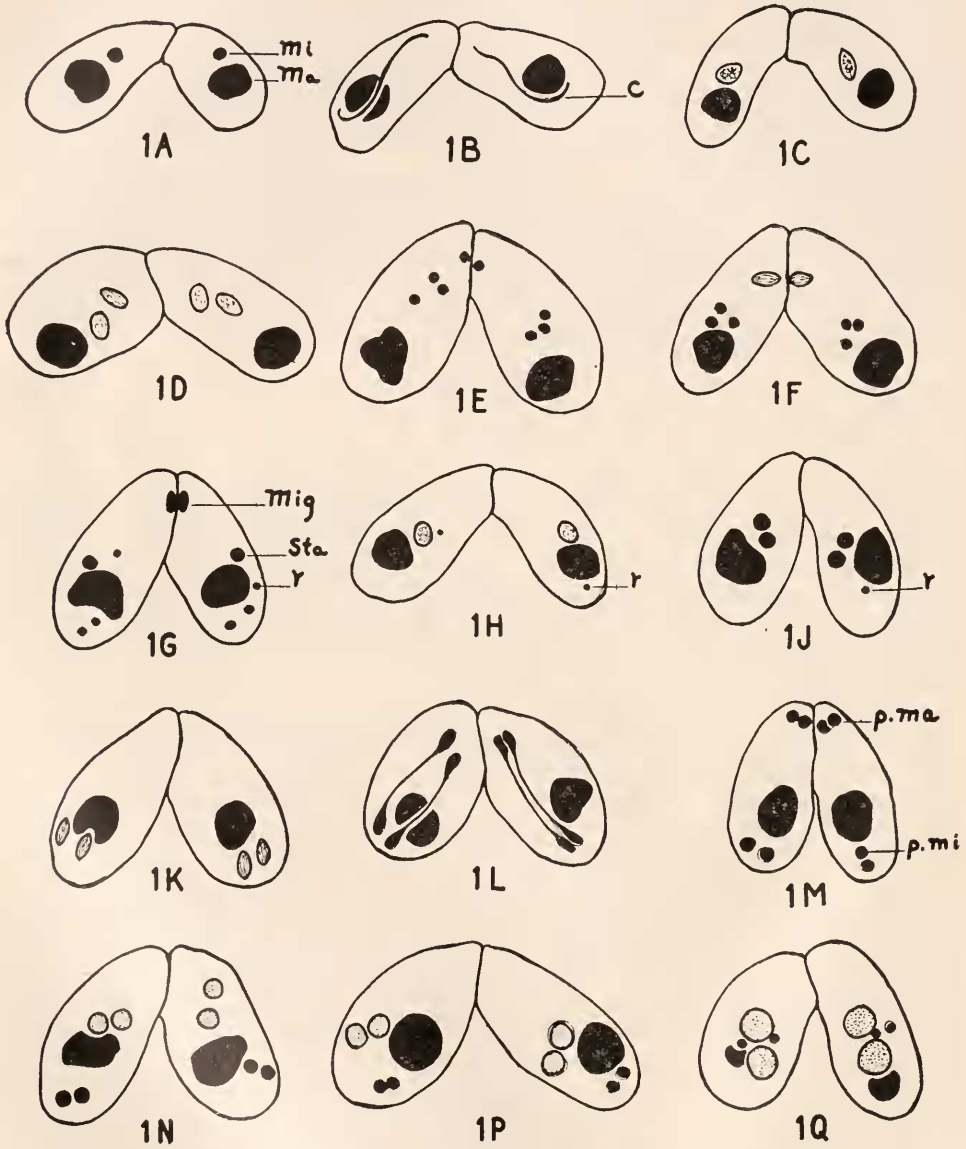

FIGURE 1. The normal sequence of nuclear changes at conjugation in *Tetrahymena sp.; mi* = micronucleus; *ma* = macronucleus; *c* = crescent; *r* = relic nuclei; *mig* = migratory nucleus; *sta* = stationary nucleus; *s* = syncaryon; *p. ma* = presumptive macronucleus; *p. mi* = presumptive micronucleus. See explanation in text.

The nucleus situated at the membrane enlarges and undergoes a third pre-zygotic division while still attached (Fig. 1F). Since the previous divisions are probably the meiotic divisions, this may be assumed to be an equational division. One of the nuclei produced by this division (the migratory nucleus) remains at its original position; the other nucleus (the stationary nucleus) moves to a region just anterior to the macronucleus (Fig. 1G) and may be distinguished from the relic nuclei by its larger size and more spherical shape. By this time these other haploid nuclei are often pycnotic and in some instances have already disintegrated.

The migratory nucleus is observed to flatten considerably (Fig. 1G) and then to round up and protrude slightly into the other cell. Eventually the migratory nuclei from the two pair members are exchanged. The fertilization nucleus is formed by the fusion, just anterior to the macronucleus, of the migratory nucleus from one cell with the stationary nucleus from the other cell. This fertilization nucleus immediately prepares to divide (Fig. 1H). Since the fertilization nucleus is formed by the union of presumably identical nuclei in the two members of a pair, it is necessary that the two exconjugants of a single pair will be alike in their genetic constitution.

Immediately following fertilization the syncaryon undergoes its first post-zygotic division while still anterior to the macronucleus. The two large conspicuous daughter nuclei (Fig. 1J) migrate posteriorly and the second and last post-zygotic nuclear division is initiated posterior to the macronucleus with the spindles oriented longitudinally in the cell (Fig. 1K). These spindles elongate until they extend nearly the entire length of the cell (Fig. 1L), and at the end of this division two nuclei are left at the extreme anterior end of the cell and two at the extreme posterior end of the cell (Fig. 1M). Those at the anterior end, the presumptive macronuclear anlagen, begin to move toward the posterior end, to enlarge and stain less intensely than before (Fig. 1N and 1P). The nuclei at the posterior end, on the other hand, remain small and become the new micronuclei.

At about this time the original macronucleus shows the first evidence of change. It loses its irregular outline, becomes spherical and deeply staining (Fig. 1P). Eventually it becomes smaller and is lost (Figs. 1Q–2B), although not invariably at the same time in both conjugants.

The pair members usually do not separate, though they may be forcibly separated during the latest stages and a few pairs separate spontaneously. Often the conjugating cells coalesce (Fig. 2C), become spherical and vacuolated, and finally lyse. The conjugants which separate likewise do not survive.

2. *Conjugation involving three cells*

Maupas (1889) reported having seen triple formations many times in *Leucophrys patula*, as well as in other ciliates, but gave no further information on either the mode of attachment or on the cytogenetic details. Triples are regularly seen in the AA cultures and may involve a small percentage of the observed conjugants. In all cases thus far observed the triples are formed by the symmetrical union of cells at the oral surfaces. No attachments at other points have been observed. In the WH *Tetrahymena* strains a different kind of triple has been found. These triples are due to the simultaneous union of two single animals to the two oral surfaces of a "double" animal. Such triples are not to be confused with the

tripolar triples reported here. A third type of triple is known in many ciliates and occurs when a third cell becomes attached in any of a variety of positions to one member of a conjugating pair. In *Paramecium bursaria,* Chen (1946) has shown that this third mate undergoes autogamy and neither receives a pronucleus from nor contributes a pronucleus to either of the other cells. Weisz (1950) reports triple formations in *Blepharisma,* some of which appear to be similar to those studied by Chen, but others of which may be of the tripolar sort described below

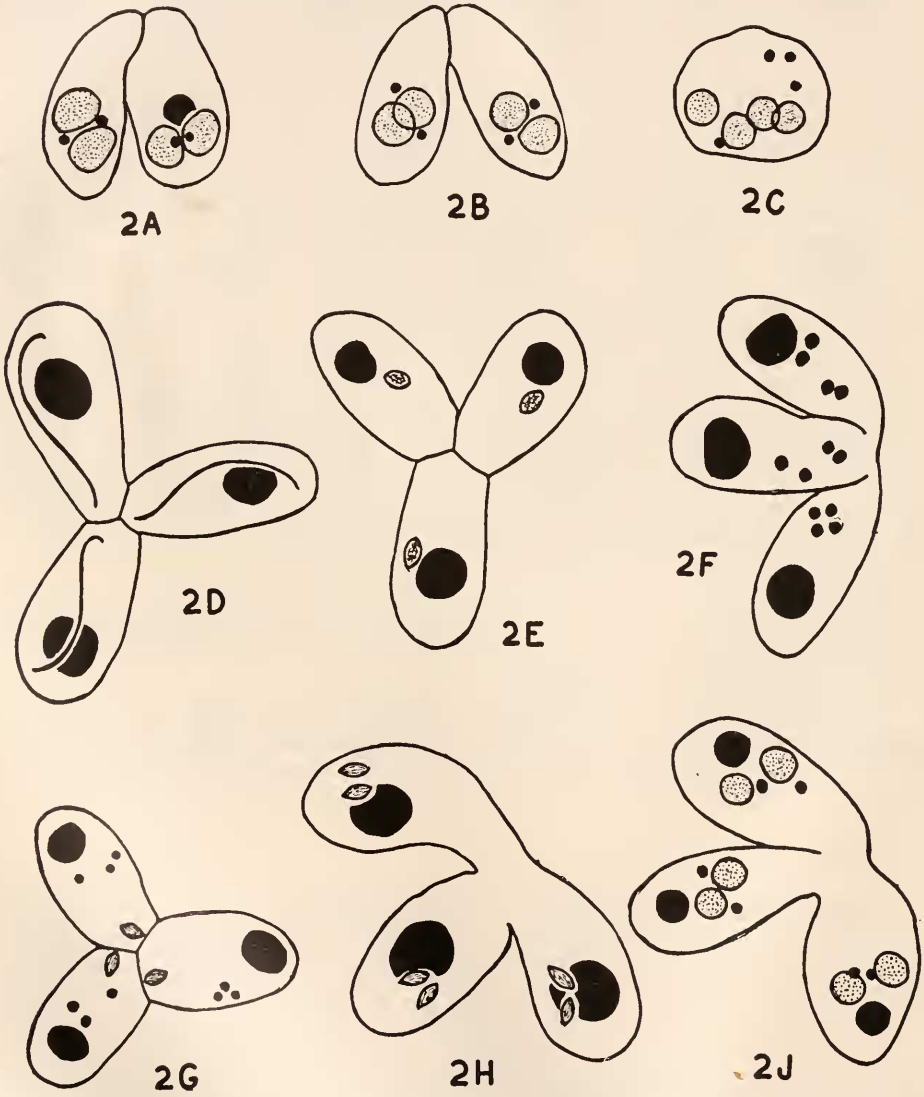

FIGURE 2. 2A–2C, terminal stages in normal conjugation. 2D–2J, conjugation in triples. See explanation in text.

for *Tetrahymena*. Since nuclear studies on the *Blepharisma* strains were not conclusive, this interpretation must remain tentative.

The pattern of nuclear behavior in the AA triples is precisely identical with that in pairs; all three cells appear to undergo a normal reorganization with the normal synchrony. Figure 2D shows a typical crescent stage; Figure 2E shows a later stage in the first pre-zygotic division. Figure 2F shows the four nuclei in each cell produced by the second pre-zygotic division. Figure 2G shows the spindles for the third pre-zygotic division. It will be noted that the dividing nuclei are all attached on what appears to be the left side of the cell (actually the right side, judging from the nuclear orientation in pairs where right and left are readily determined). It appears likely that in this case the migratory nucleus passes through the membrane to which it is attached and, hence, that each cell contributes a migratory nucleus to one cell and receives a migratory nucleus from the other cell. This is, therefore, in all probability a true tripolar fertilization and should yield different genetic results than normal conjugants. Specifically, tripolar fertilization could yield three genetically diverse cells after conjugation under some circumstances. Figure 2H shows the spindles for the second post-zygotic nuclear division and Figure 2J illustrates the stage after the new macronuclei have differentiated, but before the old macronucleus has completely disappeared.

3. *Conjugation in cells with multiple micronuclei*

Within mass cultures of several of the stocks occasional cells have been observed with multiple micronuclei. In one isolation line nearly all the cells showed two micronuclei at the time they were first stained. Subsequently the frequency of bi-micronucleate cells decreased and the culture returned to the uni-micronucleate condition. During the period when the culture possessed many bi-micronucleate cells, pairs were stained and studied.

Multiple micronuclei appear in no way to affect the behavior of the individual nuclei or to alter the consequences of conjugation. All the micronuclei originally present undergo the first and second pre-zygotic divisions. Figure 3A shows a pair in which both conjugants have two micronuclei and in which all the micronuclei are in the crescent stage. Figure 3B shows a pair in which one cell has one micronucleus and the other has two. Figures 3C–3E show the later stages in the first pre-zygotic division in conjugants with a variety of nuclear constitutions. Figure 3F shows the beginning of the second pre-zygotic division in a pair which originally consisted of a uni-micronucleate and a bi-micronucleate member. The chromatin in this stage appears as a faintly staining network, strikingly different from the chromatin in Figures 3C–3E, in which distinct chromosomes are readily seen. Figure 3G shows a pair similar to that in 3F, but after the second pre-zygotic division; eight nuclei are seen in one cell and four in the other. Figure 3H shows a pair beginning the third pre-zygotic nuclear division; the one dividing nucleus and the seven relic nuclei in each cell indicate that both cells were originally bi-micronucleate. It is observed that only one micronucleus ever undergoes the third pre-zygotic division, regardless of whether a cell contains four, eight or twelve (when the cell was originally tri-micronucleate). Following fertilization and the disintegration of the relic nuclei, no differences can be ascertained between cells

which were originally multi-micronucleate and those that were originally uni-micronucleate.

The number of micronuclei present in a cell appears to have no influence on either the probability of mating or on the kinds of matings observed. The frequency of multi-micronucleate cells in conjugation is not significantly different from the frequency of such cells in the same culture which are not conjugating at

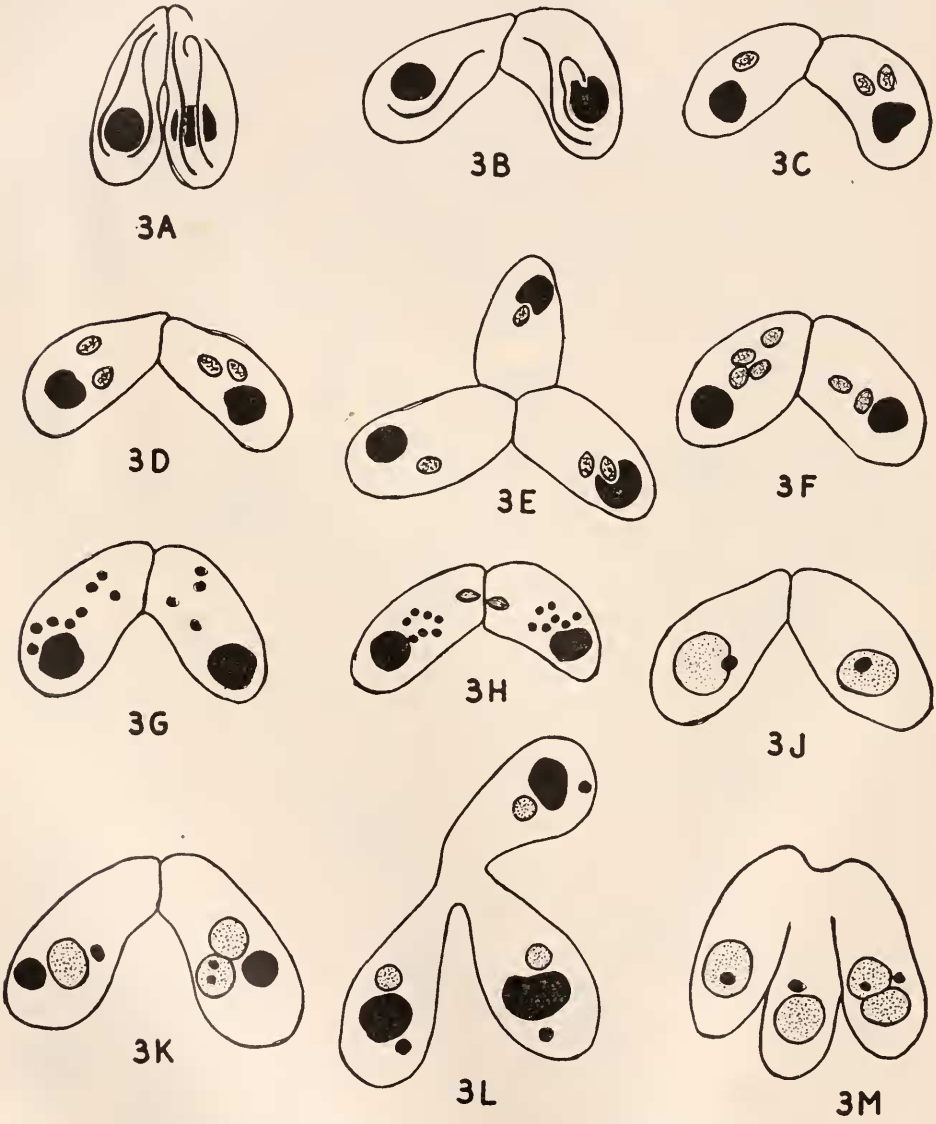

FIGURE 3. 3A–3H, conjugation in cells with multiple micronuclei. 3J–3M, a spontaneous anomaly. See explanation in text.

a particular time. The distribution of pair types (uni-micronucleate × uni-micronucleate, uni-micronucleate × bi-micronucleate, etc.) is not significantly different from the distribution expected by chance alone.

4. *A spontaneous anomaly*

Only one spontaneously occurring anomaly has been observed in the AA cultures, and this occurred in about 7% of the conjugants (45 of 608 on one slide) in a single stock (AA-1) the first time conjugation was observed; it has not been encountered since. Unfortunately, the culture in which the anomaly was found was in the later stages of conjugation, and it was not possible to determine the manner in which the abnormality developed. This anomaly is characterized by the presence at the end of conjugation of only one new macronucleus and only one new micronucleus instead of the usual two of each kind (Fig. 3J–3M). These new nuclei, both macro- and micronuclei, are clearly larger than those in normal cells. Rough measurements show a difference in volume of a factor of two compared with the nuclei in comparable stages of normal cells.

An examination of prepared slides was undertaken to determine whether pair members tended to resemble each other in respect to the number of new nuclei produced. On a particular slide 270 pairs were found, in which both conjugants were normal; 23 pairs were found with one abnormal member and 11 pairs were observed to have two abnormal members. On simple probability considerations the expected classes are 260, 42 and 2. It seems probable, therefore, that pair members tend to be alike. The significance of this observation is not clear.

Experimental

The fact that differences in the behavior of different nuclei present in the cell at the same time are correlated with the regular localization of the nuclei in specific cytoplasmic regions is sufficient to suggest that nuclear behavior is to some extent controlled by local differences in the cytoplasm. This fact alone, however, may not be considered critical evidence for such a cytoplasmic role. It is conceivable that the nuclei are self-determined to behave as they do regardless of where they are located. If, however, nuclei could be transferred from one cytoplasmic locality to another and if this relocation could be demonstrated to result in altered nuclear behavior or nuclear differentiation, the hypothesis of cytoplasmic control could be considered firmly established.

The simplest method for relocating nuclei in the cytoplasm appeared to be centrifuging. Cultures in conjugation were centrifuged in an International Clinical Centrifuge at full speed (about 5000 g) for various lengths of time; they were allowed to recover for from one to 24 hours and were then fixed and stained. Some cultures were centrifuged for ten minutes; other cultures were centrifuged for ten minutes, allowed to recover for fifteen minutes and were again centrifuged. In some instances three periods of centrifugation were used.

Cultures stained immediately after centrifugation showed that the nuclei were indeed relocated in the cytoplasm, and slides prepared at various intervals after the cells had recovered showed that alterations in nuclear behavior had been accomplished. These alterations may be described as follows.

1. *Simple mate-to-mate transfer*

The commonest abnormality observed was the transfer of part of the nuclear equipment from one cell into its mate. Observations suggest that this transfer may occur during any stage of conjugation. Figure 4A shows a pair in which the entire micronuclear material of one cell was transferred to the other cell at some stage prior to fertilization and probably prior to the attachment of a nucleus to the membranes separating the cells. One cell contains no micronuclei; the other contains seven

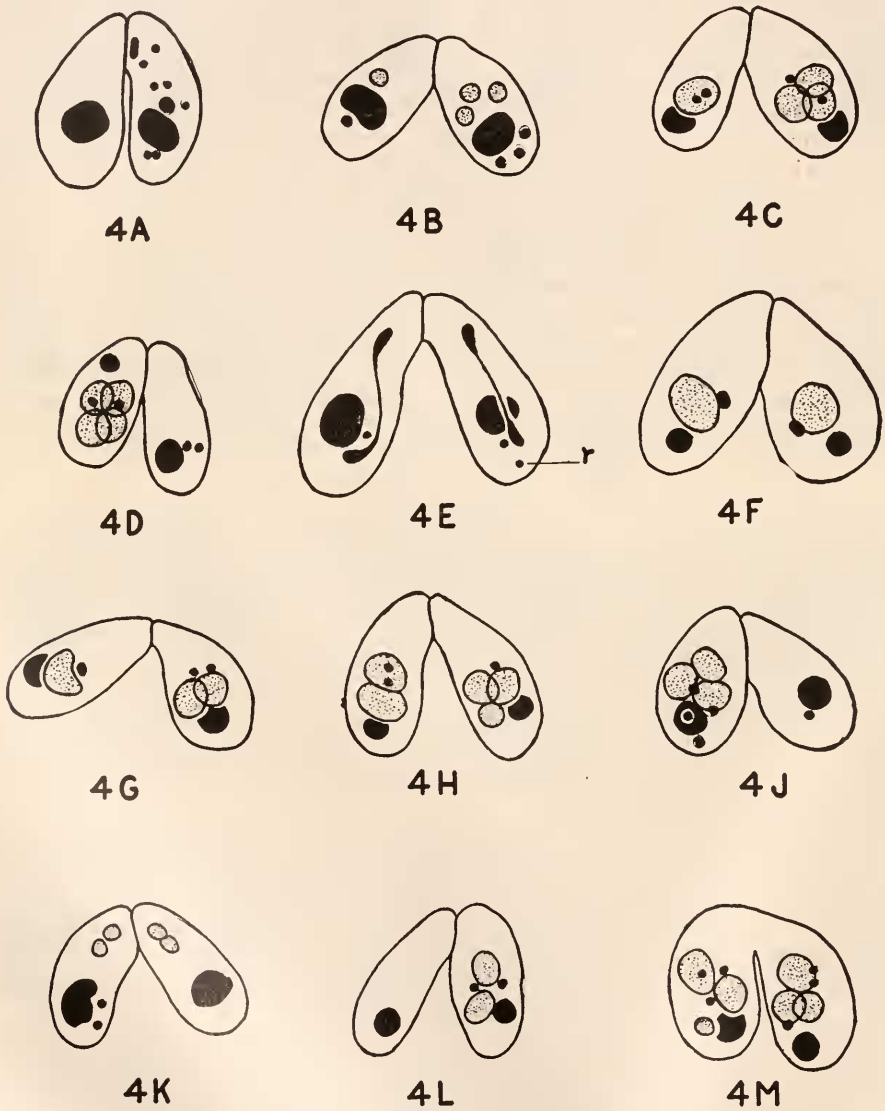

FIGURE 4. Abnormalities in conjugation induced by centrifugation. See explanation in text.

relic nuclei, a migratory nucleus and a stationary nucleus. This could be interpreted as conjugation between an amicronucleate and a bi-micronucleate cell were it not for the fact that *no* amicronucleate and *no* bi-micronucleate cells were observed elsewhere in the culture. Figure 4B is most readily interpreted as due to a mate-to-mate transfer after fertilization and presumably just after the first post-zygotic division. If this interpretation is correct, each nucleus continued to develop in its normal manner to give rise to a macronucleus and a micronucleus. Either of these cases could also be interpreted on the basis of a dual effect of the centrifugation—an elimination of nuclei from one cell and an induced extra division of nuclei in the other cell, but the usual compensation for the loss of nuclei in one cell by the addition of nuclei to its mate makes the mate-to-mate transfer interpretation more likely. In other cases where there is no compensation, the interpretation of nuclear loss or induced extra divisions may be more seriously entertained.

Mate-to-mate transfers at later stages, *i.e.*, after the differentiation of micronuclei and macronuclei, are more common. Figure 4C shows a pair in which one of the macronuclei from one cell was transferred to the other cell and Figure 4D shows a pair in which both new macronuclei were transferred from one cell into its mate. These observations show no evidence for cytoplasmic control of nuclear processes, but demonstrate clearly that relocation of nuclei in the cytoplasm does occur during centrifugation. Other, but rarer, types of abnormalities are more illuminating. Some of these are associated with mate-to-mate transfer and others are presumably due merely to alterations in the positions of the nuclei within a single cell.

2. *Alterations in the number of nuclei*

The most common abnormality observed—next to simple mate-to-mate transfer—was essentially like the spontaneous abnormality discussed above. In some cells only two new nuclei were formed in contrast to the usual four, and the new nuclei were larger than normal nuclei in comparable stages. Figure 4E shows a pair in which both members show a spindle like the typical spindle for the second post-zygotic division, but each pair shows only one such spindle. Figure 4F shows a similar pair at a later stage and Figure 4G shows a pair in which the anomaly occurred in only one cell of a pair. These abnormalities can be explained on two simple assumptions: (1) the cytoplasm at the posterior end of the cell determines that a nucleus under its influence will undergo a "final" division with a longitudinally oriented spindle, and (2) the centrifugation resulted in a fertilization nucleus being placed under this influence before it had the opportunity to undergo a normal first post-zygotic division. If this interpretation is correct, the spontaneous abnormality may be similarly explained on the basis of some unspecified environmental influence that causes the fertilization nucleus to arrive at the posterior end prematurely. An alternative interpretation would hold that the figures shown represent conjugants from which some of the nuclei have been removed by centrifugation. If this is true, the experimentally induced aberrations are not necessarily related to the spontaneous aberrations where nuclear loss seems unlikely.

3. *Alterations in nuclear development*

Another kind of aberration noted involves the change of a presumptive micronucleus into a macronucleus or of a presumptive macronucleus into a micronucleus.

Since it is not possible to follow any particular pair through conjugation, such alterations can be detected only as cells containing abnormal relative numbers of micronuclei and macronuclei. Figure 4H shows a pair which appears to satisfy expectations. One pair-member is normal, *i.e.,* it contains two micronuclei and two macronuclei. The other pair-member shows two well developed macronuclei and one typical micronucleus. The other new nucleus, presumably derived from the other presumptive micronucleus, is much larger, less deeply staining, and appears to be in the process of developing into a macronucleus. This may be interpreted as a nucleus whose developmental route was altered at a fairly late stage. Whether it would develop into a fully formed new macronucleus is of course not known. Figure 4J shows the reciprocal transformation, in this case combined with mate-to-mate transfer. One cell contains only a single micronucleus; the other contains four micronuclei and three new macronuclei. The pair as a whole has, therefore, produced five micronuclei and three macronuclei. It appears reasonable to assume that one of the presumptive macronuclei has given rise to a micronucleus. Since aberrations of this sort have been observed in the AA strains only when the cells are centrifuged, and since centrifugation certainly relocates the nuclei in the cytoplasm, it seems reasonable to assume that the relocation itself results in an alteration in nuclear development.

4. *Other alterations*

Other alterations in nuclear development have also been observed, but these are much rarer and an insufficient number of each kind has been observed to warrant extended discussion. A few of these will be illustrated. Figure 4K shows a pair in which the nuclei in one cell are normal, while the other cell contains only two nuclei—both developing as macronuclei. This can be interpreted by assuming two effects of the treatment—an alteration in nuclear number followed by the transformation of a presumptive micronucleus into a macronucleus. Since this pair had been centrifuged three times during conjugation, this interpretation does not seem improbable.

Figure 4L shows a pair consisting of one apparently normal cell and one with no new nuclei. This may be interpreted as due to the transfer of the fertilization nucleus from one cell into its mate followed by a single division of each of the nuclei. It could also be explained by a mate-to-mate transfer at an earlier stage be present, additional evidence for alteration in nuclear development is seen in the nuclei from one cell.

Figure 4M is more difficult to interpret since the total number of new nuclei in each cell is six rather than the usual four. This could be due to an extra division of one of the products of the first post-zygotic divisions in each cell. This result might also be found if this was originally a triple, the extra nuclei being derived from a third cell before it was lost. Regardless of how the multiple nuclei came to be present, additional evidence for alteration in nuclear development is seen in the small partially developed macronucleus in the left member.

Discussion

Sonneborn (1951) pointed out several instances where cytoplasmic control of nuclear behavior was indicated in the cytogenetic processes of *Paramecium.*

Particularly he suggested that cytoplasmic locations were determinative in the following instances: 1) the survival or disintegration of haploid nuclei following meiosis; 2) the differences in the behavior of the migratory and stationary nuclei; 3) the differentiation of micronuclei and macronuclei. His conclusions may be extended with little modification to *Tetrahymena* and additional instances of cytoplasmic control may also be suggested.

In proceeding to a discussion of the factors involved in nuclear behavior at conjugation in *Tetrahymena*, it is necessary to point out the general features of this behavior. These may be discussed under the following headings: nuclear migration, nuclear division and nuclear differentiation.

1. *Nuclear migration*

The various stages of nuclear reorganization are characterized by events occurring in specificially localized regions of the cytoplasm. The first question raised is whether the movements resulting in the specific localizations are autonomous, or whether the cytoplasm controls these movements to some extent. This question may not be answered with certainty but certain considerations favor the latter solution. The location of the nuclei may be understood in terms of two migrations, the first a migration toward the anterior end of the cell (toward the contact membranes) and the second a migration toward the posterior end of the cell (away from the contact membranes). Before conjugation the micronuclei occupy positions near the macronucleus, but may be either anterior or posterior to it; the first and second pre-zygotic division figures are always anterior to the macronucleus. After meiosis the nuclei continue to move anteriorly until one of the haploid nuclei attaches to the membrane between the cells. This terminates the anterior migration; all subsequent movements, except those resulting from nuclear displacement during nuclear division, are from the anterior end of the cell to the posterior end. After one nucleus attaches, the relic nuclei begin to move posteriorly and, if they do not disintegrate first, come to lie at the extreme posterior end of the cell. The fertilization nucleus also migrates posteriorly from its position in front of the old macronucleus, and the new macronuclei move posteriorly from the position where they were placed by the elongated spindles of the second post-zygotic division.

The anterior migration is initiated at the time the cells come in contact at their oral surfaces; it is terminated at the time a nucleus attaches at these same surfaces. There is thus circumstantial evidence that events occurring at the contact surface determine nuclear migrations. Several possibilities are available in regard to the nature of such events, but in the absence of further evidence, speculation appears unprofitable.

2. *Nuclear divisions*

The nuclear divisions are as follows: two meiotic divisions, a pre-zygotic equational division of one of the haploid nuclei and usually two post-zygotic divisions. The meiotic divisions, like the nuclear migrations, are initiated following the attachment of the cells. The pre-zygotic equational division is clearly related to the cytoplasmic disposition of the nuclei since it occurs only in the nuclei attached to the contact membranes. The specificity of this cytoplasmic location is shown by the

fact that attachment is always on the right side of the cell. The nature of these contact membranes, the manner in which they adhere to one another, the way they initiate the process of conjugation and control nuclear movements (if indeed they do), the manner in which they assure the transfer of presumably identical nuclei in opposite directions are all problems of importance in understanding the complex phenomena of conjugation. These problems require much further study.

The post-zygotic nuclear divisions are also controlled to some extent by the cytoplasmic conditions, but these conditions are not obviously related to the contact membranes. The first post-zygotic division occurs anterior to the old macronucleus; the second occurs posterior to the old macronucleus. Evidence has been presented which suggests that if the first post-zygotic division occurs posterior to the macronucleus, this is the final division, giving rise to a macronucleus and a micronucleus. Since the division occurring anterior to the macronucleus under normal circumstances shows a spindle oriented transversely in the cell, whereas all divisions occurring posterior to the macronucleus show spindles oriented longitudinally in the cell, the influence of this cytoplasm may lie in or be associated with its control of spindle orientation.

3. *Nuclear differentiation*

Several types of nuclear differentiation are noted during the conjugation process. The first differentiation is that of enlargement of the micronuclei prior to meiosis. Like the nuclear migrations and meiosis, this enlargement is directly or indirectly related to events occurring at the contact surfaces. The second differentiation is that occurring in the haploid nucleus attached to the membranes. The unattached nuclei disintegrate without dividing; the attached nucleus divides and both its daughter nuclei persist. Particularly it is to be noted that the daughter nucleus free in the same cytoplasm with the disintegrating nuclei does not distintegrate. It must, therefore, be different from them, though it is extremely unlikely that this difference is genetic; both types of nuclei are presumably haploid and must reasonably be expected to be alike in genetic material in many instances. The size difference between the stationary nucleus and the relic nuclei is further evidence for some kind of differentiation. Sonneborn (personal communication) suggests, on the basis of observations on *Paramecium,* that a transient cytoplasmic condition initiates the degeneration of free nuclei at a particular time, even though complete disintegration is not observed until later. According to this view, the stationary nucleus is released into the cytoplasm when the cytoplasm is no longer capable of initiating degeneration.

The differences in the behavior of the migratory and the stationary nuclei may also be ascribed to cytoplasmic relations, but it is possible that the differences are due simply to the fact that one is physically bound to the contact membranes while the other is free in the cytoplasm. One final difference between the stationary and the relic nuclei is seen in the fact that fertilization takes place between the incoming migratory nucleus and the stationary nucleus even if the relic nuclei are in the same cytoplasm. Perhaps it is premature, in the absence of genetic evidence, to conclude that the migratory nucleus *never* fuses with a relic nucleus, but this conclusion is certainly strongly indicated for other organisms that have been studied genetically (Sonneborn, 1947). It appears probable that the stationary nucleus is

attracted to or attracts the migratory nucleus and that the nuclei are so differentiated that under normal conditions the relic nuclei cannot participate in the union.

There is apparently no major difference between the fertilization nucleus and one of its daughter nuclei. Either may divide once to give rise to a macronucleus and a micronucleus. The difference in their normal behavior is apparently due to their cytoplasmic location rather than to intrinsic factors. The differences in the sizes of the macronuclei and micronuclei produced directly by these two kinds of nuclei are not understood, but may be explained on the basis of a limitation of substrate for nuclear development in the conjugating cells. According to this view, a single macronucleus and a single micronucleus in the cytoplasm have more reserves to draw on and hence develop further than would two macronuclei and two micronuclei in the same cytoplasm. Other interpretations are possible, however.

The disintegration of the macronucleus at a particular time in the conjugation cycle may also be considered a type of nuclear differentiation, but no information is available concerning the factors involved.

The final nuclear differentiation is that which distinguishes the macronuclei from the micronuclei. The evidence presented demonstrates that this differentiation of nuclei is directly related to their positions in the cytoplasm at a critical time. The conditions at the anterior end of the cell are such as to bring about the development of macronuclei; the conditions at the posterior end cause the development of micronuclei. That the nuclei developing as macronuclei are not different in their potentialities from those developing as micronuclei is shown by the fact that presumptive macronuclei may be induced to become micronuclei and presumptive micronuclei may be induced to become macronuclei by altering the positions of the nuclei in the cytoplasm. This conclusion is further supported by evidence that under some circumstances the daughter nuclei produced at the first post-zygotic division can directly differentiate as macronuclei or micronuclei; under normal circumstances each of these nuclei gives rise to one macronucleus and one micronucleus. It would be difficult to explain these results on the basis of the segregation of genetic elements.

4. *Cytoplasmic differentiation*

Evidence for nuclear differentiation of various kinds is available in the account given above. Evidence for progressive cytoplasmic differentiation through conjugation is less readily obtained, but certain observations suggest that this also plays an important role. It is known, for example, that a diploid nucleus dividing immediately anterior to the old macronucleus undergoes meiosis during the initial stages of conjugation, but undergoes mitosis after fertilization in the same position. This could be explained by some kind of nuclear differences characterizing the nuclei at the different times, but it is equally possible that the cytoplasm has been altered. Similar considerations hold in regard to the behavior of nuclei at the anterior end of the cell at different times during conjugation. The nuclei produced after the second pre-zygotic division show no evidence for developing as macronuclei, while the nuclei produced after the second post-zygotic division and placed at the anterior end do develop as macronuclei. Other examples could also be drawn in which nuclear and cytoplasmic differentiation are equally probable as an explanation for the differences in the behavior of nuclei in the same cytoplasmic re-

gions at different times. It appears probable that a progressive cytoplasmic alteration is correlated with a progressive nuclear alteration throughout conjugation and that an understanding of the process must include consideration of a complex interaction of nuclear and cytoplasmic factors.

Although the observations on *Tetrahymena* demonstrate an important influence of the cytoplasm on nuclear behavior, it cannot be concluded that the cytoplasmic conditions are not ultimately under the control of the nuclei. Observations on *Paramecium* (Sonneborn, 1951; Nanney, 1953) indicate that certain cytoplasmic conditions controlling nuclear development are determined by the nuclei and that cellular differentiation may proceed as a series of inter-determinations of the nucleus by the cytoplasm and of the cytoplasm by the nucleus. It is probable that many aspects of nuclear behavior, though immediately under the control of the cytoplasm, are ultimately traced to nuclear activity.

Summary

1. The details of the nuclear processes occurring at conjugation in certain selfing strains (AA strains) of *Tetrahymena* are presented with an experimental analysis of certain of the factors influencing nuclear behavior and nuclear differentiation.

2. While it is not possible at the present time to describe in detail the mechanisms operating to assure an orderly sequence of events, it is clear that the cytoplasm plays a critical role in directing the activities of the nuclei. This is demonstrated by two facts: that the various events are specifically localized in the cytoplasm and that experimental alterations in the positions of the nuclei result in alterations in nuclear behavior.

3. It is concluded that the entire conjugation cycle proceeds as a complex series of nucleo-cytoplasmic interactions.

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